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=> s dr#### (10a) intercalator (10a) detect?
L1 2 DR#### (10A) INTERCALATOR (10A) DETECT?

=> s l1 and immobiliz##
L2 0 L1 AND IMMOBILIZ##

=> s l1 and fluorescence
L3 0 L1 AND FLUORESCENCE

=> s l1 and nucleic acid
2 FILES SEARCHED...
L4 0 L1 AND NUCLEIC ACID

=>

=> d l1 1-2 bib ab kwic

L1 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2002 ACS
AN 1986:602797 CAPLUS
DN 105:202797
TI Kinetics and sequence specificity of drug-DNA interactions: an in vitro transcription assay
AU Phillips, Don R.; Crothers, Donald M.
CS Dep. Biochem., La Trobe Univ., Bundoora, 3083, Australia
SO Biochemistry (1986), 25(23), 7355-62
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
AB An assay to detect the DNA-sequence specificity of drug-binding sites, and the kinetics of dissocn. of drug from those sites, under conditions involving active transcription of the DNA is described. Specific transcriptional blockage sites were **detected** in the presence of actinomycin D [50-76-0] and a bis-**intercalator**, bis(anthracycline); the rate of RNA chain growth past the **drug** -binding sites yielded the rate of dissocn. of drug from these sites. Rate consts. for dissocn. from the whole promoter fragment measured by the detergent sequestration method were faster than the rate detd. for the dissocn. from the specific transcriptional blockage site. However, the absence of significant blockage at other drug-binding sites implied much more rapid drug dissocn. from those sites in the transcriptional complex. Apparently, transcriptional blockage results from a DNA-sequence-dependent interaction of the drug-DNA complex with RNA polymerase [9014-24-8]; the sequences that are effective for blockage appear to be GpC for actinomycin and (CpA)3 for a bis(daunomycin) compd. Transcriptional inhibition may in general show greater sequence specificity than is exhibited by simple binding.
AB An assay to detect the DNA-sequence specificity of drug-binding sites, and

the kinetics of dissocn. of drug from those sites, under conditions involving active transcription of the DNA is described. Specific transcriptional blockage sites were **detected** in the presence of actinomycin D [50-76-0] and a bis-**intercalator**, bis(anthracycline); the rate of RNA chain growth past the **drug** -binding sites yielded the rate of dissocn. of drug from these sites. Rate consts. for dissocn. from the whole promoter fragment measured by the detergent sequestration method were faster than the rate detd. for the dissocn. from the specific transcriptional blockage site. However, the absence of significant blockage at other drug-binding sites implied much more rapid drug dissocn. from those sites in the transcriptional complex. Apparently, transcriptional blockage results from a DNA-sequence-dependent interaction of the drug-DNA complex with RNA polymerase [9014-24-8]; the sequences that are effective for blockage appear to be GpC for actinomycin and (CpA)3 for a bis(daunomycin) compd. Transcriptional inhibition may in general show greater sequence specificity than is exhibited by simple binding.

L1 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1987:87480 BIOSIS
DN BA83:46058
TI KINETICS AND SEQUENCE SPECIFICITY OF DRUG-DNA INTERACTIONS AN IN-VITRO TRANSCRIPTION ASSAY.
AU PHILLIPS D R; CROTHERS D M
CS DEPARTMENT OF BIOCHEMISTRY, LA TROBE UNIVERSITY, BUNDOORA, VICTORIA, 3083, AUSTRALIA.
SO BIOCHEMISTRY, (1986) 25 (23), 7355-7362.
CODEN: BICHAW. ISSN: 0006-2960.
FS BA; OLD
LA English
AB An assay has been developed to detect the DNA sequence specificity of drug binding sites, and the kinetics of dissociation of drug from those sites, under conditions involving active transcription of the DNA. Specific transcriptional blockage sites were **detected** in the presence of actinomycin D and a bis-**intercalator**, bis(anthracycline); the rate of RNA chain growth past the **drug** binding sites yields the rate of dissociation of drug from these sites. Rate constants for dissociation from the whole promoter fragment measured by the detergent sequestration method were found to be significantly faster than the rate determined for dissociation for the specific transcriptional blockage site. However, the absence of significant blockage at other drug binding sites implies much more rapid drug dissociation from those sites in the transcriptional complex. We conclude that transcriptional blockage results from a DNA sequence-dependent interaction of the drug-DNA complex with RNA polymerase; the sequences that are effective for blockage appear to be GpC for actinomycin and (CpA)3 for a bis(daunomycin) compound. Transcriptional inhibition may in general show greater sequence specificity than is exhibited by simple binding.
AB . . . of dissociation of drug from those sites, under conditions involving active transcription of the DNA. Specific transcriptional blockage sites were **detected** in the presence of actinomycin D and a bis-**intercalator**, bis(anthracycline); the rate of RNA chain growth past the **drug** binding sites yields the rate of dissociation of drug from these sites. Rate constants for dissociation from the whole promoter. . .

=> s (dried or drying) (10a) intercalatro (10a) detect? (10a) (nucleic acid or DNA or RNA)
L5 0 (DRIED OR DRYING) (10A) INTERCALATRO (10A) DETECT? (10A) (NUCLEIC ACID OR DNA OR RNA)

=> s (dried or drying)(10a)intercalator(10a)detect?(10a)(nucleic acid or DNA or RNA)
L6 0 (DRIED OR DRYING)(10A) INTERCALATOR(10A) DETECT?(10A)(NUCLEIC

ACID OR DNA OR RNA)

=> s (dried or drying)(10a)intercalator#(10a)detect?
L7 0 (DRIED OR DRYING)(10A) INTERCALATOR#(10A) DETECT?

=> s (dried or drying)(10a)intercalator#
L8 0 (DRIED OR DRYING)(10A) INTERCALATOR#

=> s intercalator#(10a)detect?
L9 94 INTERCALATOR#(10A) DETECT?

=> s l9 and (nucleic acid or DNA or RNA)
2 FILES SEARCHED...
L10 94 L9 AND (NUCLEIC ACID OR DNA OR RNA)

=>

=> s l10 and immobiliz?
L11 16 L10 AND IMMOBILIZ?

=> s l11 and (dried or drying or dry)
L12 1 L11 AND (DRIED OR DRYING OR DRY)

=> d l12 bib ab kwic

L12 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
AN 2002:160169 CAPLUS
DN 136:229065
TI Method for detecting/quantitating **nucleic acid** by probe hybridization and **dry** fluorometry in microarray application
IN Yamamoto, Nobuko; Okamoto, Hisashi; Suzuki, Tomohiro
PA Canon Inc., Japan
SO Jpn. Kokai Tokkyo Koho, 12 pp.
CODEN: JKXXAF
DT Patent
LA Japanese
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 2002065275	A2	20020305	JP 2000-263507	20000831

AB A method is provided for detecting/quantitating a multiple-stranded **nucleic acid** possessing a specific base sequence using a fluorescent dye by **dry** fluorometry. The method comprises the following steps: (a) a fluorescent dye capable of emitting fluorescence or enhancing fluorescence in the presence of the multiple-stranded **nucleic acid**, and maintaining the fluorescence emission in a **dry** state is added to a sample soln. as an object for detection or quantitation. (b) A known amt. of the sample soln. to which the fluorescent dye has been added is placed on a clean baseplate, and is **dried**. (c) The fluorescence from the **dried** sample is measured, and the multiple-stranded **nucleic acid** in the sample soln. is detected/quantitated based on the measurement results obtained. Provided is a method of attaching oligonucleotide probes to a solid support with high d. and efficiency in matrix or array format for microarray application using a reaction between maleimido group on the glass plate and thiol group on the oligonucleotide. Maleimido group can be introduced by first introducing an amino group to the glass substrate and reacting it with succinimidyl-4-(p-maleimidophenyl)butyrate. Oligonucleotides are attached to the glass substrate by reaction of their amino group with the epoxy group of the glass substrate. Samples are spotted by ink-jet method. Detn. of **nucleic acid** by **detecting** PCR amplification product using a fluorescent **intercalator**, 2-methyl-4,6-bis(4-N,N-dimethylaminophenyl)pyrylium

iodide (P2), which does not fluoresce in the free state but shows strong fluorescence when reacted with dsDNA (.lambda.ex 580 nm, .lambda.em 640 nm), in proportion to the amt. of dsDNA, and its deriv., is presented. The use of P2 enables precise and selective detection of the amplification product of PCR reactions, by adding the dye compd. directly to a PCR reaction mixt. without sepg. the amplification product from the primers and template **nucleic acid**.

TI Method for detecting/quantitating **nucleic acid** by probe hybridization and **dry** fluorometry in microarray application

AB A method is provided for detecting/quantitating a multiple-stranded **nucleic acid** possessing a specific base sequence using a fluorescent dye by **dry** fluorometry. The method comprises the following steps: (a) a fluorescent dye capable of emitting fluorescence or enhancing fluorescence in the presence of the multiple-stranded **nucleic acid**, and maintaining the fluorescence emission in a **dry** state is added to a sample soln. as an object for detection or quantitation. (b) A known amt. of the sample soln. to which the fluorescent dye has been added is placed on a clean baseplate, and is **dried**. (c) The fluorescence from the **dried** sample is measured, and the multiple-stranded **nucleic acid** in the sample soln. is detected/quantitated based on the measurement results obtained. Provided is a method of attaching oligonucleotide probes to a solid support with high d. and efficiency in matrix or array format for microarray application using a reaction between maleimido group on the glass plate and thiol group on the oligonucleotide. Maleimido group can be introduced by first introducing an amino group to the glass substrate and reacting it with succinimidyl-4-(p-maleimidophenyl)butyrate. Oligonucleotides are attached to the glass substrate by reaction of their amino group with the epoxy group of the glass substrate. Samples are spotted by ink-jet method. Detn. of **nucleic acid** by **detecting** PCR amplification product using a fluorescent **intercalator**, 2-methyl-4,6-bis(4-N,N-dimethylaminophenyl)pyrylium iodide (P2), which does not fluoresce in the free state but shows strong fluorescence when reacted with dsDNA (.lambda.ex 580 nm, .lambda.em 640 nm), in proportion to the amt. of dsDNA, and its deriv., is presented. The use of P2 enables precise and selective detection of the amplification product of PCR reactions, by adding the dye compd. directly to a PCR reaction mixt. without sepg. the amplification product from the primers and template **nucleic acid**.

ST **nucleic acid** probe array solid support maleimido thiol reaction; hybridization assay silane solid surface **immobilization** **nucleic acid**; multiple stranded **DNA** **dry** fluorometry dye; PCR amplification product detection fluorescence intercalation; pyrylium dye P2 PCR product detection

IT Fluorescent dyes
Fluorometry
Glass substrates
Immobilization, molecular
(attaching **nucleic acid** probes to a solid support via maleimido-thiol reaction for microarray application)

IT Nucleic acids
RL: ANT (Analyte); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study)
(attaching **nucleic acid** probes to a solid support via maleimido-thiol reaction for microarray application)

IT Probes (**nucleic acid**)
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); CPS (Chemical process); PEP (Physical, engineering or chemical process); ANST (Analytical study); BIOL (Biological study); PROC (Process); USES (Uses)
(attaching **nucleic acid** probes to a solid support via maleimido-thiol reaction for microarray application)

IT Printing (nonimpact)
(bubble jet, use in spotting samples; attaching **nucleic**

acid probes to a solid support via maleimido-thiol reaction for microarray application)

IT Functional groups
(maleimido, reaction with thiol group; attaching **nucleic acid** probes to a solid support via maleimido-thiol reaction for microarray application)

IT **DNA** microarray technology
Nucleic acid hybridization
(method for detecting/quantitating **nucleic acid** by probe hybridization and **dry** fluorometry in microarray application)

IT Epoxy group
(reaction with amino group; attaching **nucleic acid** probes to a solid support via maleimido-thiol reaction for microarray application)

IT Sulfhydryl group
(reaction with maleimido group; attaching **nucleic acid** probes to a solid support via maleimido-thiol reaction for microarray application)

IT Amino group
(reaction with succinimidyl-4-(p-maleimidophenyl)butyrate; attaching **nucleic acid** probes to a solid support via maleimido-thiol reaction for microarray application)

IT Ink-jet printing
(use in spotting samples; attaching **nucleic acid** probes to a solid support via maleimido-thiol reaction for microarray application)

IT 110-94-1, Glutaric acid
RL: RCT (Reactant); RACT (Reactant or reagent)
(anhyd.; attaching **nucleic acid** probes to a solid support via maleimido-thiol reaction for microarray application)

IT 13558-31-1 151921-86-7 321351-91-1, 2-(3-Carboxypropyl)-4,6-bis(4-N,N-dimethylaminophenyl)pyrylium 321351-95-5
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(attaching **nucleic acid** probes to a solid support via maleimido-thiol reaction for microarray application)

IT 55750-63-5, N-(6-Maleimidocaproyloxy)succinimide 79886-55-8
RL: CPS (Chemical process); PEP (Physical, engineering or chemical process); RCT (Reactant); PROC (Process); RACT (Reactant or reagent)
(attaching **nucleic acid** probes to a solid support via maleimido-thiol reaction for microarray application)

IT 2124-31-4
RL: RCT (Reactant); RACT (Reactant or reagent)
(attaching **nucleic acid** probes to a solid support via maleimido-thiol reaction for microarray application)

IT 403070-22-4 403070-23-5
RL: PRP (Properties)
(unclaimed sequence; method for detecting/quantitating **nucleic acid** by probe hybridization and **dry** fluorometry in microarray application)

=>

09/764050

WEST

Freeform Search

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Term: detect$ near5 fluorescence near5 dry$

Display: 10 **Documents in Display Format:** - **Starting with Number** 1

Generate: ○ Hit List ◉ Hit Count ○ Side by Side ○ Image

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Search History

DATE: Tuesday, August 20, 2002 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L16	detect$ near5 fluorescence near5 dry$	9	L16
L15	L11 and immobiliz$6	1	L15
L14	L13 and solid	0	L14
L13	L11 and glass	0	L13
L12	L11 and resin	0	L12
L11	L10 and (nucleic acid or DNA or RNA)	2	L11
L10	L9 and intercalator$1	2	L10
L9	dry$ near5 detect$	6102	L9
L8	L7	0	L8
L7	dry$ near5 detect$ near5 intercalator	0	L7
L6	L3 and ethidium	1	L6
L5	L3 and yoyo-1	0	L5
L4	L3 and pyrylium	0	L4
L3	L1 and (glass or resin)	1	L3
L2	L1 and glass or resin	1511817	L2
L1	6401267.pn.	1	L1

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 9 of 9 returned.

☐ 1. 6372446 . 03 Aug 99; 16 Apr 02. Method of selectively determining a fungal biomass. Miller; Morten, et al. 435/7.31; 435/34 435/4 435/7.1 435/7.4 435/7.72 435/7.9. G01N033/53.

☐ 2. 6231338 . 09 May 00; 15 May 01. Method and apparatus for the detection of carious activity of a carious lesion in a tooth. de Josselin de Jong; Elbert, et al. 433/29; 433/215 433/229. A61C001/00 A61C003/00.

☑ 3. 6077669 . 04 Nov 97; 20 Jun 00. Kit and method for fluorescence based detection assay. Little; Michael C., et al. 435/6; 435/91.2 435/968. C12Q001/68 C12P019/34 G01N033/53.

☐ 4. 5527708 . 02 May 95; 18 Jun 96. Sensitive and highly specific quantitative fluorometric assay for creatinine in biological fluids. Blass; Karl G.. 436/98; 422/56 422/82.08 436/106 436/164 436/169 436/172 436/96. G01N033/00.

☐ 5. 4062948 . 11 Sep 75; 13 Dec 77. Dihydromocimycin antibiotics. Vos; Cornelis, et al. 514/23; 435/118 435/886 536/53 546/116 546/282.1. A61K031/70 C07H007/00.

☐ 6. JP 08094543 A . 22 Sep 94. 12 Apr 96. METHOD FOR DETECTING MICRODEFECT. YOSHIDA, TOSHIAKI, et al. G01N021/91;.

☐ 7. JP 59157546 A . 28 Feb 83. 06 Sep 84. FLUORESCENT X-RAY SPECTROGRAPHIC ANALYSIS OF SOLUTION SAMPLE. MARUYAMA, HIDEO, et al. 378/44. G01N023/223;.

☐ 8. US 6077669 A EP 915173 A2 AU 9889603 A CA 2249638 A1 JP 11225799 A . New method for real-time fluorescence-detection assays useful for detecting nucleic acids from pathogens in samples from patients. LITTLE, M C, et al. C12N015/09 C12P019/34 C12Q001/68 G01N021/78 G01N033/50 G01N033/52 G01N033/53.

☐ 9. US 5723976 A EP 703446 A1 JP 08094543 A TW 283259 A . Detecting minute defects in encapsulated electronic component - by immersion in soln. of water soluble fluorescent substance and observing image of component to detect fluorescence and its cessation. NORIMATSU, T, et al. G01N001/30 G01N021/64 G01N021/91 H01L021/66.

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Term	Documents
FLUORESCENCE.DWPI,EPAB,JPAB,USPT.	53088
FLUORESCENCES.DWPI,EPAB,JPAB,USPT.	355
DETECT$	0
DETECT.DWPI,EPAB,JPAB,USPT.	920402
DETECTA.DWPI,EPAB,JPAB,USPT.	10
DETECTAB.DWPI,EPAB,JPAB,USPT.	1
DETECTABALE.DWPI,EPAB,JPAB,USPT.	5
DETECTABE.DWPI,EPAB,JPAB,USPT.	6
DETECTABEL.DWPI,EPAB,JPAB,USPT.	3
DETECTABIE.DWPI,EPAB,JPAB,USPT.	5
DETECTABILI.DWPI,EPAB,JPAB,USPT.	4
(DETECT$ NEAR5 FLUORESCENCE NEAR5 DRY$).USPT,JPAB,EPAB,DWPI.	9

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L7: Entry 5 of 8 File: USPT Jul 31, 2001

DOCUMENT-IDENTIFIER: US 6268131 B1
TITLE: Mass spectrometric methods for sequencing nucleic acids

Detailed Description Text (70):
Once transcription is complete, the nucleic acids can be analyzed by any of a variety of means including, for example, spectrometric techniques such as UV/VIS, IR, fluorescence, chemiluminescence, or NMR spectroscopy, mass spectrometry, or other methods know in the art, or combinations thereof. Preferred mass spectrometer formats include the ionization (I) techniques, such as matrix assisted laser desorption (MALDI), continuous or pulsed electrospray (ESI) and related methods (e.g. lonspray or Thermospray), or massive cluster impact (MCI); these ion sources can be matched with detection formats including linear or reflectron time-of-flight (TOF), single or multiple quadruple, single or multiple magnetic sector, Fourier Transform ion cyclotron resonance (FTICR), ion trap, and combinations thereof to yield a hybrid detector (e.g., ion-trap/time-of-flight). For ionization, numerous matrix/wavelength combinations (MALDI) or solvent combinations (ESI) can be employed.

Detailed Description Text (105):
After depositing the sample arrays onto the surface of the substrate, the arrays can be analyzed using any of a variety of means (e.g., spectrometric techniques, such as UV/VIS, IR, fluorescence, chemiluminescence, NMR spectrometry or mass spectrometry. For example, subsequent to either dispensing process, sample loaded substrates can be placed onto a MALDI-TOF source plate and held there with a set of beveled screw mounted polycarbonate supports. In one practice, the plate can be transferred on the end of a probe to be held onto a 1.mu.m resolution, 1" travel xy stage (Newport) in the source region of a time-of-flight mass spectrometer. It will be apparent to one of ordinary skill in the art that any suitable mass spectrometry tool can be employed with the present invention without departing from the scope thereof.

Detailed Description Text (193):
The solution was allowed to dry at ambient temperature and thereafter a 6-nl aliquot of water was added to each location using a piezoelectric pipette to resuspend the dried matrix-DNA complex, such that upon drying at ambient temperature the matrix-DNA complex forms a uniform crystalline surface on the bottom surface of each location.

Detailed Description Text (207):
In vitro transcription of the nicked DNA template was carried out in 20 .mu.l reactions of 40 mM Tris-HCl (pH 7.0), 6 mM MgCl.sub.2, 2 mM spermidine, 10 mM NaCl, 10 mM dithiothreitol, 1 unit/.mu.l RNasin (Promega), 5 mM rNTP, 5 .mu.Ci (.alpha.-32P) rCTP, 1 unit/.mu.l SP6 RNA polymerase (Amersham, Arlington Heights, Ill.) at 37.degree. C. for 30 minutes. Abortive and full length RNA transcripts were separated by gel electrophoresis and quantified by measuring the radioactivity of individual RNA fragments by drying the polyacrylimide gel and measuring the radioactivity as compared to a known standard using a Phospholmager (Molecular Dynamics, Inc.). The efficiency of full-length RNA transcription of a nicked DNA template was calculated as a percentage the moles of full length RNA transcribed from a DNA template containing no nicks. The nick by pass efficiency of a nicked DNA template was calculated as a percentage of the moles full-length RNA transcript and the moles of RNA transcript stalled at the nick.

Other Reference Publication (69):
Thuong and Asseline, Oligonucleotides attached to intercalators, photoreactive and cleavage agents, Oligonucleotides and Analogues: A Practical Approach, Eckstein, edr., Oxford University Press Ch. 12, pp. 283-308 (1991).

Other Reference Publication (139):

Green and Jorgenson et al., "Variable-wavelength on-column fluorescence detector for open-tubular zone electrophoresis", J. Chromatography 352:337-343 (1986).

Other Reference Publication (156):
Jett et al., "High-Speed DNA Sequencing: An Approach Based Upon fluorescence Detection of Single Molecules", J. Bio Strut & Dynam. 7(2):301-09 (1989).

Other Reference Publication (159):
Jurinke C. et al., "Recovery of nucleic acids from immobilized biotin-streptavidin complexes using ammonium hydroxide and applications in MALDI-TOF mass spectrometry", Anal. Chem. 69(5):904-10 (1997).

Other Reference Publication (219):
Singh et al., "Oligonucleotides, part 5 +: synthesis and fluorescence studies on DNA oligomers d(AT).sub.5 containing adenines covalently linked at C-8 with dansyl fluorophore", Nucleic Acids Res. 18(11):3339-3345 (1990).

Other Reference Publication (225):
Smith et al., Fluorescence detection in automated DNA sequence analysis, Nature 321:674-679 (1986).

Generate Collection

L16: Entry 3 of 9 File: USPT Jun 20, 2000

DOCUMENT-IDENTIFIER: US 6077669 A
TITLE: Kit and method for fluorescence based detection assay

Detailed Description Text (4):
However, when real time fluorescence detection assays are formatted with reagents in a dry form to be rehydrated, the rehydration of the dry fluorescently labeled reagent causes an interfering fluorescence background which is not constant and changes irreproducibly. Thus, this interfering background masks the specific fluorescence change and cannot be corrected for by numerical methods. Therefore, the method and kit of the present invention are designed to eliminate this cause of interfering fluorescence background by postponing initiation of the detection reaction which produces the desired change in fluorescence signal until after rehydration of the fluorescently labeled reagent.

Detailed Description Text (37):
Comparative Example Using a Kit and Method Wherein Rehydration of Dry Fluorescence Detector Probe and Amplification of a Target Analyte Occurs in a First Vessel of Such Kit During a First Step of Such Method

End of Result Set

☐ Generate Collection

L6: Entry 1 of 1 File: USPT Jun 11, 2002

DOCUMENT-IDENTIFIER: US 6401267 B1
TITLE: Methods and compositions for efficient nucleic acid sequencing

US PATENT NO. (1):
6401267

Brief Summary Text (46):
In the methods of the invention, the oligonucleotides of the first set may be attached to a solid support, i.e. immobilized, by any of the methods known to those of skill in the art. For example, attachment may be via addressable laser-activated photodeprotection (Fodor et al., 1991; Pease et al., 1994). One generally preferred method is to attach the oligos through the phosphate group using reagents such as nucleoside phosphoramidite or nucleoside hydrogen phosphorate, as described by Southern & Maskos (PCT Patent Application WO 90/03382, incorporated herein by reference), and using glass, nylon or teflon supports. Another preferred method is that of light-generated synthesis described by Pease et al. (1994; incorporated herein by reference). One may also purchase support bound oligonucleotide arrays, for example, as have been offered for sale by Affymetrix and Beckman.

Detailed Description Text (27):
Support bound oligonucleotides may be prepared by any of the methods known to those of skill in the art using any suitable support such as glass, polystyrene or teflon. One strategy is to precisely spot oligonucleotides synthesized by standard synthesizers. Immobilization can be achieved using passive adsorption (Inouye & Hondo, 1990); using UV light (Nagata et al., 1985; Dahlen et al., 1987; Morriey & Collins, 1989) or by covalent binding of base modified DNA (Keller et al., 1988; 1989); all references being specifically incorporated herein.

Detailed Description Text (34):
In more detail, to use this method, a support, such as a glass plate, is derivatized by contact with a mixture of xylene, glycidoxypropyltrimethoxysilane, and a trace of diisopropylethylamine at 90.degree. C. overnight. It is then washed thoroughly with methanol, ether and air-dried. The derivatized support is then heated with stirring in hexaethyleneglycol containing a catalytic amount of concentrated sulfuric acid, overnight in an atmosphere of argon, at 80.degree. C., to yield an alkyl hydroxyl derivatized support. After washing with methanol and ether, the support is dried under vacuum and stored under argon at -20.degree. C.

Detailed Description Text (35):
Oligonucleotide synthesis is then performed by hand under standard conditions using the derivatized glass plate as a solid support. The first nucleotide will be a 3'-hydrogen phosphate, used in the form of the triethylammonium salt. This method results in support bound oligonucleotides of high purity.

Detailed Description Text (36):
An on-chip strategy for the preparation of DNA probe arrays may be employed. For example, addressable laser-activated photodeprotection may be employed in the chemical synthesis of oligonucleotides directly on a glass surface, as described by Fodor et al. (1991), incorporated herein by reference. Probes may also be immobilized on nylon supports as described by Van Ness et al. (1991); or linked to teflon using the method of Duncan & Cavalier (1988); all references being specifically incorporated herein.

Detailed Description Text (46):
A suitable synthetic support is one consisting of a 5.1.times.7.6 cm glass substrate

prepared by cleaning in concentrated NaOH, followed by exhaustive rinsing in water. The surfaces would then be derivatized for 2 hr with a solution of 10% (vol/vol) bis(2-hydroxyethyl)aminopropyltriethoxysilane (Petrarch Chemicals, Bristol, Pa.) in 95% ethanol, rinsed thoroughly with ethanol and ether, dried in vacuo at 40.degree. C., and heated at 100.degree. C. for 15 min. In such studies, a synthesis linker would be attached by reacting derivatized substrates with 4,4'-dimethoxytrityl (DMT)-hexaethyloxy-O-cyanoethyl phosphoramidite.

Detailed Description Text (123):
An alternative luminescent detection procedure involves the use of fluorescent or chemiluminescent reporter groups attached to the target molecules. The fluorescent labels can be attached covalently or through interaction. Fluorescent dyes, such as ethidium bromide, with intense absorption bands in the near UV (300-350 nm) range and principal emission bands in the visible (500-650 nm) range, are most suited for the CCD devices employed since the quantum efficiency is several orders of magnitude lower at the excitation wavelength than at the fluorescent signal wavelength.

CLAIMS:

26. The method of claim 19, wherein said immobilized oligonucleotide are attached to a glass, polystyrene or teflon solid support.

41. The kit of claim 37, wherein the oligonucleotide probes are attached to a glass, polystyrene or teflon solid support.

59. The plate of claim 49, wherein said oligonucleotide probes are attached to a plurality of supports made of nylon, glass, polystyrene or teflon, and said plurality of supports are immobilized on said microchips.

65. The microchip of claim 64, wherein said oligonucleotide probes are attached to a plurality of supports made of nylon, glass, polystyrene or teflon, and said plurality of supports are immobilized on said microchips.

78. The method of claim 77, wherein said oligonucleotide probes are attached to a plurality of supports made of nylon, glass, polystyrene or teflon, and said plurality of supports are immobilized on said microchips.

☐ Generate Collection

L7: Entry 2 of 8 File: USPT Jun 11, 2002

DOCUMENT-IDENTIFIER: US 6401267 B1
TITLE: Methods and compositions for efficient nucleic acid sequencing

Brief Summary Text (45):
Next one would detect the secondary complexes by detecting the presence of the label and identify sequences of length F+P from the nucleic acid fragments in the secondary complexes by combining the known sequences of the hybridized immobilized and labelled probes. Stretches of the sequences of length F+P that overlap would then be identified, thereby allowing the complete nucleic acid sequence of the molecule to be reconstructed or assembled from the overlapping sequences determined.

Detailed Description Text (56):
The M nucleoside analogue was designed to maximize stacking interactions using aprotic polar substituents linked to heteroaromatic rings, enhancing intra- and inter-strand stacking interactions to lessen the role of hydrogen bonding in base-pairing specificity. Nichols et al. (1994) favored 3-nitropyrrole 2'-deoxyribonucleoside because of its structural and electronic resemblance to p-nitroaniline, whose derivatives are among the smallest known intercalators of double-stranded DNA.

Detailed Description Text (99):
Cate et al. (1991) describe the use of oligonucleotide probes directly conjugated to alkaline phosphatase in combination with a direct chemiluminescent substrate (AMPPD) to allow probe detection. Alkaline phosphatase may be covalently coupled to a modified base of the oligonucleotide. After hybridization, the oligo would be incubated with AMPDD. The alkaline phosphatase enzyme breaks AMPDD to yield a compound that produces fluorescence without excitation, i.e., a laser is not needed. It is contemplated that a strong signal can be generated using such technology.

Detailed Description Text (125):
For probe immobilization on the detector, hybridization matrices may be produced on inexpensive SiO.sub.2 wafers, which are subsequently placed on the surface of the CCD following hybridization and drying. This format is economically efficient since the hybridization of the DNA is conducted on inexpensive disposable SiO.sub.2 wafers, thus allowing reuse of the more expensive CCD detector. Alternatively, the probes can be immobilized directly on the CCD to create a dedicated probe matrix.

CLAIMS:

13. A method of nucleic acid sequencing comprising the steps of:

(a) fragmenting the nucleic acid to be sequenced to provide nucleic acid fragments of length T;

(b) preparing an array of immobilized oligonucleotide probes of known sequences and length F and a set of labeled oligonucleotide probes in solution of known sequences and length P, wherein F+P.English Pound.T;

(c) contacting said array of immobilized oligonucleotide probes with said nucleic acid fragments under hybridization conditions effective to allow the formation of primary complexes with hybridized, completely complementary sequences of length F and non-hybridized fragment sequences of length T-F;

(d) contacting said complexes with said set of labeled oligonucleotide probes under hybridization conditions effective to allow only the formation of secondary complexes with hybridized, completely complementary sequences of length F and immediately adjacent hybridized, completely complement sequences of length P;

(e) covalently bonding said labeled oligonucleotide probes to said immediately adjacent immobilized oligonucleotide probes;

(f) detecting said secondary complexes by detecting the presence of the label;

(g) identifying sequences of length F+P from the nucleic acid fragments in said secondary complexes by combining the known sequences of the hybridized immobilized and labeled probes;

(h) determining stretches of said sequences of length F+P that overlap; and

(i) assembling the complete nucleic acid sequence from said overlapping sequences.

19. A method of nucleic acid sequencing comprising the steps of:

(a) fragmenting the nucleic acid to be sequenced to provide intermediate length nucleic acid fragments;

(b) contacting an array of immobilized small oligonucleotide probes of known sequences with said nucleic acid fragments under hybridization conditions effective to allow only those fragments with a completely complementary sequence to hybridize to a probe, thereby forming primary complexes wherein the fragment has hybridized and non-hybridized sequences;

(c) contacting said primary complexes with a set of labeled small oligonucleotide probes in solution of known sequences under hybridization conditions effective to allow only those probes with completely complementary sequences to hybridize to a non-hybridized fragment sequence, thereby forming secondary complexes wherein the fragment is hybridized to an immobilized probe and a labeled probe;

(d) covalently bonding said labeled oligonucleotide probes to said immediately adjacent immobilized oligonucleotide probes;

(e) removing from said secondary complexes labeled probes that are not covalently bonded to an immobilized probe, thereby forming covalently bonded complexes;

(f) detecting said covalently bonded complexes by detecting the presence of the label;

(g) identifying sequences from-the nucleic acid fragments in said covalently bonded complexes by combining the known sequences of the hybridized immobilized and labeled probes;

(h) determining stretches of said sequences that overlap; and

(i) assembling the complete nucleic acid sequence from said overlapping sequences identified.